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**Original Article**

# The possible protective effect of vanillic acid against thioacetamide-induced hepatotoxicity in rats

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## A B S T R A C T



**Abstract:** Vanillic acid (VA) is one of the most abundant phenolic acids in plants and might be found in high concentrations in vanilla beans and sugar cane molasses. The present study investigated the possible mechanisms underlying the hepatoprotective effect of vanillic acid on thioacetamide (TAA)-induced acute liver failure (ALF).

The hepatoprotective effect of vanillic acid was evaluated by the assay of liver function parameters as serum aminotransferases (ALT and AST) as well as total bilirubin level (TB), and oxidative stress markers as hepatic malondialdehyde (MDA), reduced glutathione (GSH) content, and catalase activity (CAT), in addition to inflammatory markers as nuclear factor kappa B (NF-κB), tumor necrosis factor-α (TNF-α) and nitric oxide (NO). Furthermore histopathological study of the liver was carried out.

Preadministration of vanillic acid significantly lowered the elevated activity of serum AST, ALT enzymes and TB level and restored the hepatic abnormal levels of enzymatic antioxidants and MDA as well as decreased the elevated levels of inflammatory markers induced by TAA administration in a dose-related manner. The chemical pathological changes were consistent with histopathological observations.

These results indicate that vanillic acid could be useful in protection against TAA-induced ALF. Its significant hepatoprotective activity could be due to its antioxidative activity in addition to its anti-inflammatory properties..

**Key Words:** Thioacetamide; hepatoprotective; vanillic acid; acute liver failure

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## 1. INTRODUCTION

Acute liver failure (ALF) is a devastating clinical syndrome characterized by abrupt onset of severe acute liver injury, occurring in a patient with no preexistent liver disease, causing multi organ failure with extremely high mortality rates **(Fix, 2013)**. The causes of ALF encompass a wide variety of toxic, viral, metabolic, vascular and autoimmune insults to the liver **(Wang *et al.*, 2013)**. Liver transplantation remains the only curative treatment option. However, this treatment option is limited by scarcity of donor organs, lifelong immunosuppressant and high costs **(Morabito and Adebayo, 2014)**. Therefore, a better understanding of the path physiological features of ALF can lead to find therapeutic approaches capable of delaying the progression of the disease.

Excessive oxidative stress and inflammation are well established to play a central role in the pathogenesis of ALF regarding the initiation and the progression of the disease. Moreover, they are inextricably linked as one begins the other is amplified, and they work together to aggravate liver injury **(Jaeschke and Ramachandran, 2011; Zimmermann *et al.*, 2012)**. Reactive oxygen species (ROS) cause tissue injury directly by affecting tissue lipids, proteins and DNA as well as indirectly by triggering inflammation, leucocytes accumulation in liver tissue with massive release of inflammatory mediators that induce further ROS liberation, amplifying the inflammatory response and exacerbating liver tissue damage (**Jaeschke, 2011; Bosoi and Rose, 2013)**.

Thioacetamide (TAA), a potent hepatoxicant, has been widely used to induce ALF in several previous studies. The main very reactive products of TAA are TAA S-oxide and TAA-S dioxides are (**Hajovsky *et al.*, 2012)**. These highly reactive metabolites exert hepatotoxicity by binding to liver macromolecules, leading to excessive generation of reactive oxygen species (ROS) and induction of oxidative stress. Moreover, TAA-S-dioxide is responsible for the stimulation of nuclear factor kappa B (NF-κB) and the resultant expression of pro-

inflammatory mediators such as TNF-α and iNOS from activated hepatic macrophages, which are thought to potentiate TAA-induced acute hepatic damage, finally directing to severe hepatocellular necrosis (**Akhtar and Sheikh, 2013 )**.

Phenolic acids are plant metabolites widely spread throughout various plants **(Kim *et al.,* 2010)**. Vanillic acid (VA) is a phenolic acid found at high concentrations in sugar cane molasses and vanilla beans (**Guimares *et al*., 2007; Sinha *et al.,* 2008)**. VA showed cytoprotective effects in multiple preclinical disease models due to its promising antioxidant activity and anti-inflammatory effects **(Huang *et al.,* 2008; Kim *et al.,* 2010; prince *et al.,* 2011)**.

Silymarin (SM), a known hepatoprotective agent is a flavonolignan obtained from the seeds of silybum marianum **(Kshirsagar et al., 2009)**. The hepatoprotective effect of silymarin is based on its antioxidant property; by scavenging prooxidant free radicals, it inhibits lipid peroxidation and increases the intracellular concentration of tripeptide GSH. In addition, it stimulates RNA and protein synthesis leading to faster regeneration after liver injury **(Jensen *et al*., 2003)**. Moreover, SM modulates inflammation in hepatic tissue by mast cell stabilization, inhibition of neutrophil migration and leukotrienes production. Moreover, SM suppresses both NF-κB DNA binding activity and its dependent gene expression **(Kim *et al*., 2013)**.

Therefore, the present study aimed to investigate the possible mechanisms underlying the hepatoprotective effect of VA on TAA- induced ALF in rats, by studying its effect on several oxidative stress and anti-inflammatory markers, and whether it could ameliorate the progression of the disease. In addition, the achieved protective effect will be compared with silymarin as a reference drug.

## 2. MATERIALS AND METHODS

### 2.1.Marerials

#### *2.1.1 Drugs and chemicals*

Thioacetamide (TAA) and vanillic acid (VA) were obtained from (Sigma, St Louis, MO, USA). Silymarin (SM) was supplied by CID pharmaceutical company (Cairo, Egypt). All the chemicals were analytical grade and purchased from Sigma-Aldrich Co. (St. Louis, MO).

#### *2.1.2 Animals*

Sixty adult male Sprague–Dawley rats, weighing 150-180 g, were purchased from Helwan Farm for Laboratory Animals. The animals were maintained at standard housing conditions and fed standard pellet diet and water *ad libitum*. The animal experiments described later were approved by the Ethics Committee, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

### *2.2 Methods*

#### *2.2.1 Experimental protocol*

The animals were divided into 6 groups, 10 per each group, as follows:

**Control group**: Rats received 10% tween 80 solution orally by gavage for 15 days. On day 14$^{th}$, 15$^{th}$ they received an intraperitoneal injection of saline (0.3 ml/100mg) after one hour from giving tween 80.

**VA group**: Rats received vanillic acid (VA) (200mg/kg) dissolved in 10% tween 80 solution orally by gavage for 15 days. On day 14$^{th}$, 15$^{th}$ they received an intraperitoneal injection of saline (0.3 ml/100mg) after one hour from giving VA.

**TAA group**: Rats received 10% tween 80 solution orally by gavage for 15 days. On day 14$^{th}$, 15$^{th}$ they received an intraperitoneal injection of Thioacetamide (TAA) (200mg/kg) **(Mehul and Varsha, 2012)** after one hour from giving tween 80.

**VA (100 mg/kg) + TAA group**: Rats received VA (100 mg/kg) dissolved in 10% tween 80 solution orally by gavage for 15 days. On day 14$^{th}$, 15$^{th}$ they received an intraperitoneal injection of TAA (200mg/kg) after one hour from giving VA.

**VA (200 mg/kg) + TAA group**: Rats received VA (200 mg/kg) dissolved in 10% tween 80 solution orally by gavage for 15 days. On day 14$^{th}$, 15$^{th}$ they received an intraperitoneal injection of TAA (200mg/kg) after one hour from giving VA.

**SM (50 mg/kg) + TAA group**: Rats received Silymarin (SM) (50mg/kg) dissolved in 10% tween 80 solution orally by gavage for 15 days. On day 14$^{th}$, 15$^{th}$ they received an intraperitoneal injection of TAA (200mg/kg) after one hour from giving SM.

After 24 hour of the TAA or saline injection, blood was collected from animals by retroorbital puncture and then animals were sacrificed by cervical dislocation, the liver was rapidly removed and washed in ice-cold saline solution. A part was homogenized in phosphate buffer saline (0.1 M PBS, pH 7.4). Then the homogenates were centrifuged at 10,000 rpm for 30 min at 4˚ C, and supernatants were stored at -70˚ C until biochemical assays could be performed. The other part of liver was stored in 10 % neutral buffered formalin for histopathological study.

#### *2.2.2 Determination of liver function tests*

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities as well as serum total bilirubin (TB) level were measured as indicators of hepatic injury using standard diagnostic kits (Quimica Clinica Aplicada S.A., Spain).

### *2.2.3 Determination of oxidative stress markers*

Hepatic malondialdehyde (MDA) level was determined according to **Mihara and Uchiyama (1978)**. Reduced glutathione (GSH) level was measured using Ellman reagent **Ellman (1959)**. As well as, catalase (CAT) activity was measured according to **Aebi (1984)**.

### *2.2.4. Determination of total protein*

The protein content was measured using bovine serum albumin (BSA) as a standard according to **Lowry et al. (1951)**.

### *2.2.5 Determination of inflammatory markers*

Liver content of nuclear factor kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α) were determined by enzyme linked immunosorbent assay kits specific for rats according to the manufacturer's instruction (Uscn Life Science & Technology Company, Missouri, USA). Hepatic nitric oxide (NO) was measured colorimetrically as described by **Green et al. (1982)**.

### *2.2.6 Histopathological examination*

Paraffin tissue blocks were prepared for sectioning and the obtained sections were stained by hematoxylin & eosin stain then examined through the light electric microscope.

### *2.2.7 Statistical analysis*

All the results are expressed as Mean ± SEM, the results were analyzed for statistical significance by one-way ANOVA followed by **Tukey-Kramer (1949)** as post ANOVA.

## 3. RESULTS

### *3.1 Effect of VA on liver functions in TAA induced ALF in rats*

Intraperitoneal injection of TAA (200 mg/kg) significantly increased serum ALT, AST activities and TB level by 3.4, 1.4 and 3.1 folds, respectively, as compared to control group. While oral pre-administration of VA at doses (100 and 200 mg/kg) significantly reduced their levels by 24%, 19%, 44% and by 41%, 31%, 61%, respectively, as compared to TAA group. In addition, oral pre-administration of SM at dose (50 mg/kg) significantly reduced their levels by 32%, 29% and 53%, respectively. The protection effect of VA was acting in a dose dependent manner significantly in the level of ALT and TB only. Additionally, pretreatment with VA at both doses revealed no significant difference in ALT, AST activities and TB level as compared to SM group (Table 1).

### *3.2 Effect of VA on oxidative stress markers in TAA induced ALF in rats*

Comparing to control group TAA administration showed a significant increase in MDA level by 1.5 fold. While oral administration of VA (100 and 200 mg/kg) or SM prior to TAA intoxication significantly decreased its level by 25%, 38% and 37%, respectively, as compared to TAA group (Figure 1). On the other hand TAA administration caused a significant fall in GSH level and CAT activity by 45% and 32%, respectively, as compared to control group. Preadministration of VA (100 mg/kg) failed to significantly increased GSH level but succeeded significantly in increasing CAT activity by 30% as compared to TAA group. However VA (200 mg/kg) and SM significantly increased GSH level by 65% and 60%, respectively, as compared to TAA group. They also induced a significant increase in CAT activity by 57% and 69%, respectively, as compared to TAA group. The protection effects of VA in oxidative stress markers were significantly in a dose dependent manner. Additionally, pretreatment with VA (200 mg/kg) showed no significant difference in MDA, GSH level and CAT activity as compared to silymarin (Figure 2, 3).

### *3.3 Effect of VA on inflammatory markers in TAA induced ALF in rats*

Induction of hepatotoxicity by TAA significantly increased hepatic NF-κB, TNF-α and NO levels by 18, 1.1 and 1.5 folds respectively as compared to control group. Pretreatment with vanillic acid (100 mg/kg) showed a significantly decreasing in their levels by 26%, 20% and 18% respectively as compared to TAA group. While, pretreatment with vanillic acid (200 mg/kg) significantly decreased their levels by 44%, 48% and 28%, respectively as compared to TAA group. Furthermore, pre-administration of Silymarin showed a significantly decreasing in their levels by 41%, 52% and 23% respectively as compared with TAA group. The protection effects of VA against inflammatory markers appear significantly in a dose dependent manner in NF-κB and TNF-α level. Additionally, pretreatment with VA (200 mg/kg) showed no significant difference in NF-κB and TNF-α levels as compared to silymarin (Figure 4, 5, 6).

### *3.4 Histopathological findings*

Histopathological examination of liver sections from control group showed normal liver architecture, of the portal artery (pa) and surrounding hepatocytes (h) (figure 7A). Similarly, no histopathological changes were recorded in liver sections of rats treated with VA (200mg/kg) (figure 7B). Liver sections of thioacetamide group showed massive inflammatory

cell infiltration (m) surrounding the central vein (cv) as well as in between the necrosis of hepatocytes (n) (figure 7C). While liver sections of rats pre-treated with VA (100mg/kg) + TAA showed mild inflammatory cells infiltration (m) surrounding the central vein (cv), with degeneration in the hepatocytes (d) (figure 7D). As well as liver sections of rat liver tissue pre-treated with VA (200mg/kg) + TAA showed almost normal histological structure of the central vein (cv) and surrounding hepatocytes (h) (figure 7E). Liver sections of rat liver tissue pre-treated with SM (50 mg/kg) showed mild inflammatory cells infiltration in the portal area, with degeneration in the hepatocytes(d) (figure 7F).

**Table 1. Effect of vanillic acid (VA) on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities and total bilirubin (TB) level in thioacetamide (TAA) induced acute liver failure in rats.**

| Groups | ALT (U/L) | AST (U/L) | TB (mg/dl) |
|---|---|---|---|
| Control (vehicle) | 28.92±1.05 | 57.75±1.42 | 0.16±0.008 |
| VA (200 mg/kg) | 26.39±1.09 | 60.09±3.60 | 0.15±0.006 |
| TAA (200mg/kg) | 129.36±7.46 [a,b] | 138.73±8.16 [a,b] | 0.66±0.037 [a,b] |
| VA (100 mg/kg) +TAA | 98.88±4.10 [a,b,c] | 112.25±6.97 [a,b,c] | 0.37±0.025 [a,b,c] |
| VA (200mg/kg) + TAA | 75.91±3.98 [a,b,c,d] | 95.44±4.90 [a,b,c] | 0.26±0.014 [a,b,c,d] |
| SM (50 mg/kg) + TAA | 87.49±5.38 [a,b,c] | 98.04±7.40 [a,b,c] | 0.31±0.018 [a,b,c] |

Results are expressed as the mean ±SEM (n=10) in each group.
a: significantly different from control group at P<0.05
b: significantly different from VA group (200mg/kg) at P<0.05
c: significantly different from TAA group at P<0.05
d: significantly different from VA (100 mg/kg) at P<0.05

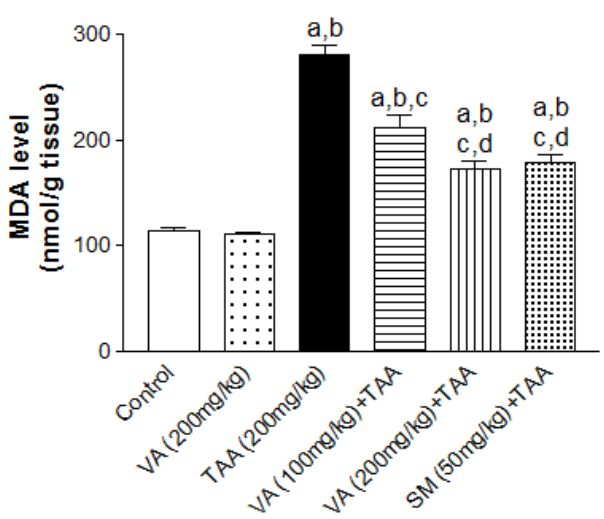


**Figure 1. Effect of vanillic acid (VA) on lipid peroxidation (MDA) level in thioacetamide (TAA) induced acute liver failure in rats.**
Results are expressed as the mean ±SEM (n=10) in each group.
a: significantly different from control group at P<0.05.
b: significantly different from VA group at P<0.05.
c: significantly different from TAA group at P<0.05.
d: significantly different from VA (100mg/kg) + TAA group at P<0.05.

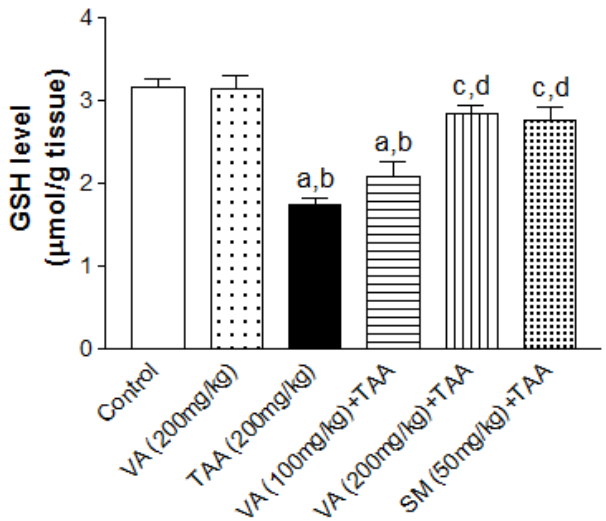


**Figure 2. Effect of vanillic acid (VA) on reduced glutathione (GSH) level in thioacetamide (TAA) induced acute liver failure in rats.**
Results are expressed as the mean ±SEM (n=10) in each group.
a: significantly different from control group at P<0.05.
b: significantly different from VA group at P<0.05.
c: significantly different from TAA group at P<0.05.
d: significantly different from VA (100mg/kg) + TAA group at P<0.05.

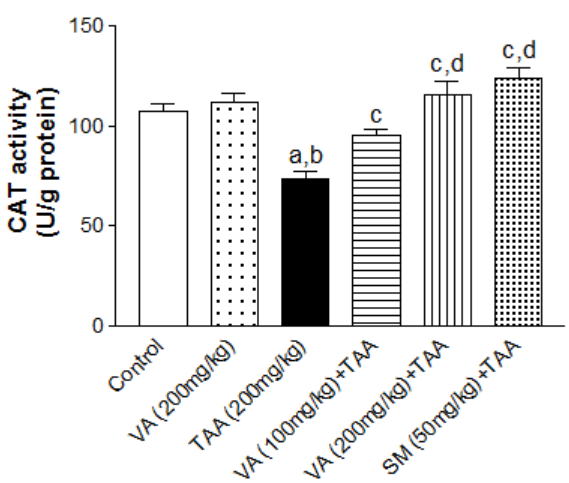


**Figure 3. Effect of vanillic acid (VA) on catalase enzyme (CAT) activity in thioacetamide (TAA) induced acute liver failure in rats.**
Results are expressed as the mean ±SEM (n=10) in each group.
a: significantly different from control group at P<0.05.
b: significantly different from VA group at P<0.05.
c: significantly different from TAA group at P<0.05.
d: significantly different from VA (100mg/kg) + TAA group at P<0.05.

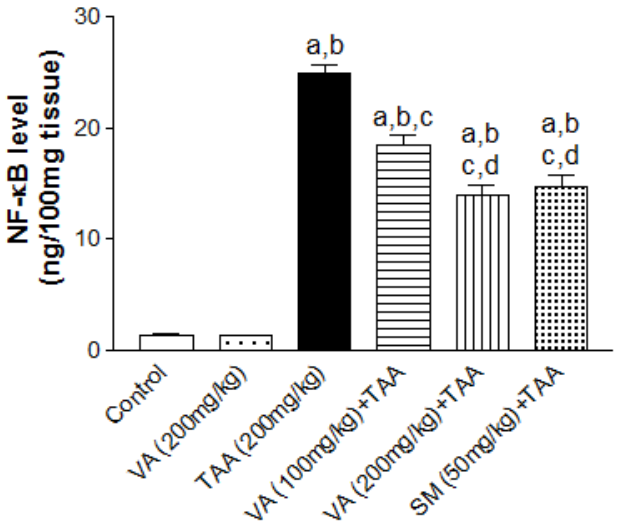


**Figure 4. Effect of vanillic acid (VA) on nuclear factor kappa B (NF-κB) level in thioacetamide (TAA) induced acute liver failure in rats.**
Results are expressed as the mean ±SEM (n=10) in each group.
a: significantly different from control group at P<0.05.
b: significantly different from VA group at P<0.05.
c: significantly different from TAA group at P<0.05.
d: significantly different from VA (100mg/kg) + TAA group at P<0.05.

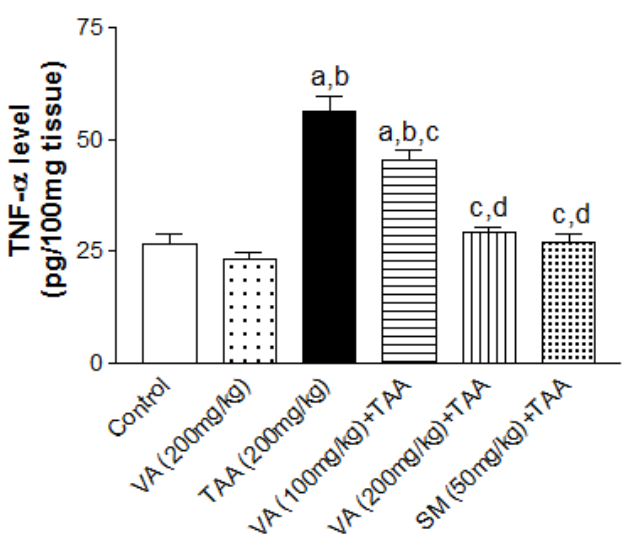


**Figure 5. Effect of vanillic acid (VA) on tumor necrosis factor-alpha (TNF-α) level in thioacetamide (TAA) induced acute liver failure in rats.**
Results are expressed as the mean ±SEM (n=10) in each group.
a: significantly different from control group at P<0.05.
b: significantly different from VA group at P<0.05.
c: significantly different from TAA group at P<0.05.
d: significantly different from VA (100mg/kg) + TAA group at P<0.05.

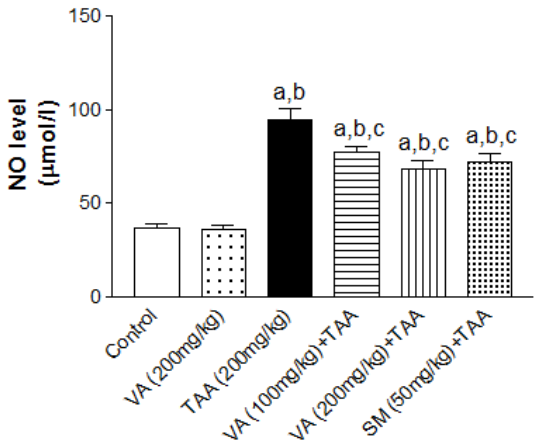


**Figure 6. Effect of vanillic acid (VA) on nitric oxide (NO) level in thioacetamide (TAA) induced acute liver failure in rats.**
Results are expressed as the mean ±SEM (n=10) in each group.
a: significantly different from control group at P<0.05.
b: significantly different from VA group at P<0.05.
c: significantly different from TAA group at P<0.05.

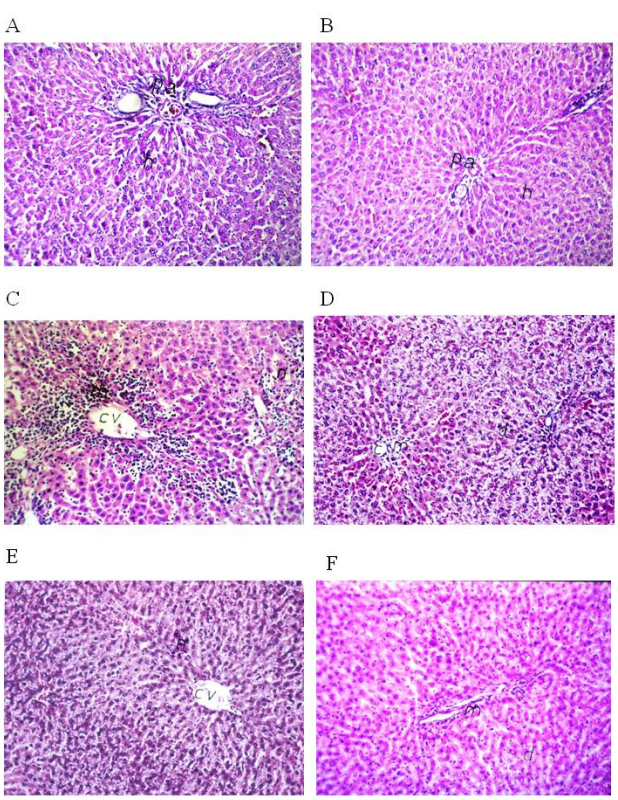


**Figure 7. Hematoxilin- and Eosin-stained sections showing the effect of vanillic acid (VA) on histological rats liver changes in thioacetamide (TAA) induced acute liver failure in rats.**
Original magnification, x40, (A): Control group; (B): VA (200mg/kg) group; (C): TAA (200mg/kg) group; (D): VA (100mg/kg) + TAA group; (E): VA (200mg/kg) + TAA group; (F): SM (50mg/kg) + TAA group.
(pa): portal artery; (h): hepatocytes; (m): infiltration; (cv): central vein; (d): degeneration; (n): necrosis.

## 4. DISCUSSION

Results of the present study revealed that acute TAA intoxication caused a significant elevation in serum hepatotoxicity markers including ALT, AST and total bilirubin. The abnormal high level of these liver function tests are the consequence of TAA induced liver dysfunction and denotes the death to the hepatic cells which leads to leakage of these cellular enzymes into the circulation and increased biliary pressure. This was consistent with the histopathological liver necrosis findings observed in our study. These results are in harmony with the findings of **Lim *et al*. (2011)**.

On the other hand, pretreatment with different doses of VA (100, 200mg/kg) showed a marked decrease in the previously mentioned hepatotoxicity markers in dose related manner and improved the liver architecture. These effects may be through stabilization of the hepatic cell plasma membrane. Moreover, effective control of bilirubin level by vanillic acid points towards an early improvement in the secretary mechanism of the hepatic cell. The aforementioned data are in harmony with those obtained by other investigators **(Itoh *et al*., 2010)**.

Under normal condition, the body has a potent antioxidant defense system, fighting against excessive generation of ROS. However, when either excessive ROS is generated or the antioxidant defense is damaged, oxidative stress occurs, leading to structural and functional injury **(Ha *et al.,* 2004)**.

In the present study, TAA administration showed a significant increase in MDA level accompanied by a significant decrease in GSH level. These results are in consonance with **Anbarasu *et al*. (2012)** who reported a decrease in GSH concentration accompanied by an increase in the content of lipid peroxidation after TAA administration. Excess generation of ROS in TAA treated rats can be attributed to increase xanthine oxidase (XO) activity **(Shapiro *et al.,* 2006).** XO enzyme generated partially reduced oxygen moieties such as superoxide anion radical and hydrogen peroxide as by-products, which can further be converted to a highly reactive hydroxyl radical by Fenton reaction resulting in initiation, propagation of lipid peroxidation, and alteration of cell membrane integrity leading to necrotic cell death **(Winterbourn and Sutton, 1986)**. Therefore the observed decreasing in reduced glutathione level might be due to increase its utilization in protecting thiol containing proteins from lipid peroxides and from other reactive oxygen species.

Pre-administration of VA in two doses (100 & 200 mg /kg) to TAA-induced rats in the present study showed a significant change in hepatic MDA and GSH level in a dose dependent manner moreover VA (200 mg/kg) succeeded in normalize GSH level. The current results was in harmony with **Kumar *et al.* (2012)** who reported that the antioxidant properties of VA is closely related to the ability of VA to scavenge free radicals in different *invitro* assay. In addition, the *invivo* study of **prince *et al.* (2011)** reported that VA provoked cardioprotective effect through inhibition of heart lipid peroxidation in isoproterenol induced cardio toxic rats. The scavenging activities of VA might be due to the active hydrogen donating ability of hydroxyl substitutions, being a radical target which thereby prevented the membrane from free radical attack and inhibit the lipid peroxidation and allowing GSH to accumulate in the cell/tissue and hence, a high level of GSH is detected as stated by **Kumar *et al.* (2012).**

Catalase is considered as one of the important enzymes in the supportive team of defense against reactive oxygen species (ROS) where it catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules thus protects the liver tissues from highly reactive hydroxyl radicals **(Lobo *et al.,* 2010).** The present study showed a significant decrease in hepatic CAT activity after TAA intoxication. This suggests an overconsumption of the enzyme related to increased production of $H_2O_2$. While, pre-administration of VA at both doses showed a significant elevation in CAT activity in TAA – toxicated rats, in a dose dependent manner. Moreover VA (200 mg/kg) succeeded to normalized CAT activity. This result evidently confirms the antioxidant property of VA against oxygen free radicals and adds further support to the *in vivo* work of **Kumar et al. (2011)** who stated that the antioxidant ability of VA to reduce accumulation of free radicals such as superoxide, hydrogen peroxide, and hydroxyl radicals generated during lipid peroxidation could restore the antioxidant machinery in the plasma of L-NAME-induced hypertensive rats.

Inflammation is a crucial feature of ALF, NF-κB plays a central role in the inducible expression of many inflammatory genes during the immune response (**Xiao and Ghosh, 2005)**. Activation of NF-κB results in production and secretion of a myriad of proinflammatory mediators such as Tumor-necrosis factor alpha (TNF-α), chemokines, adhesion molecules and inducible inflammatory enzymes such as inducible NO synthases and cyclooxygenase-2, **(Bonizzi and Karin, 2004;Chen *et al*., 2008)**.

In this context, assessment of different inflammatory markers revealed that TAA intoxication induced a significant increase in hepatic NF-κB level together with significant elevation in tissue levels of the TNF-α and NO indicating amplified inflammatory response. Acute Liver damage caused by TAA may be due to a direct effect by activation of NF-κB, or by an

indirect response in which ROS generated by TAA activate NF-κB **(Bruck *et al*., 2002)**.

It was previously established that TNF-α can turn up NF-κB activity both in target liver cells and in macrophages **(Barnes and Karin, 1997)**. As TNF-α could trigger degradation of inhibitor kappa β (Iκβ) kinase enzyme, then activated NF-κB is translocated to the nucleus to activate related gene **(Hoffman *et al*., 2002; Nakano *et al*., 2006).** Thus, a vicious cycle is established in the hepatocytes: TNF-α promotes NF-κB activation and NF-κB enhance further production of additional TNF-α leading to perpetuation and amplification of the distributive inflammatory process **(Muriel, 2009)**. Furthermore, oxidative stress is a powerful stimulant for the pro-inflammatory cytokines **(Mak and Newton, 2001)** which can result in direct cytotoxicity and further oxidative stress.

On the other hand TAA injection activated NF-κB which induce the expression of iNOS enzyme and lead to excessive NO production **(wang *et al*., 2004; Shapiro et al., 2006)**. The current result was also in accordance with **Moustafa *et al*. (2012)** who stated that acute TAA injection significantly increased NO content in liver tissue.

In the present study, pretreatment with VA significantly reduced hepatic NF-κB activation and hence inhibited the downstream inflammatory cascade as evidenced by significantly decreasing the hepatic tissue levels of TNF-α and NO in a dose related manner. This was associated with reduced severity of hepatic inflammation and liver cell injury in the histopathological finding which confirms the anti-inflammatory properties of VA.

The suppressive effect of VA on NF-κB level in the present study could be due to its antioxidant property which has been proven in the current study. This was in harmony with **Kim *et al*. (2010)** who showed that VA exerted an inhibitory effect on the inflammatory response in ulcerative colitis through the regulation of NF-κB p65 activation. The mechanistic pathway could be explained by **(Kim *et al*., 2011)** who reported that vanillic acid inhibited lipopolysaccharide-induced NF-κB activation by suppressing IκB-α degradation and Rel/p65 translocation in mouse peritoneal macrophages. Indeed, this is the first study that deals with the *invivo* effects of VA on NF-κB activation and the subsequent proinflammatory cascade during ALF.

It is therefore acceptable to suggest that the inhibition of TAA-induced oxidative stress and NF-κB activation by VA may be associated with reduced gene transcription and suppressed the formation of potent proinflammatory factors such as TNF-α. This observation is in agreement with the previous report of **Prince *et al*. (2011)** who demonstrated that VA pretreatment could decrease the expression of the pro-inflammatory cytokine gene of TNF-α, reduce oxidative stress in the myocardium of isoproterenol induced cardiotoxic rats.

Furthermore **Kim *et al*. (2011)** proved that VA decreased NO production associated with inhibition of NF-κB activation So by similar mechanism , VA suppress NF-κB activation in TAA toxicated liver tissue lead to decreased expression of iNOS and hence subsequent NO production. In addition, owing to the potent free radical scavenging properties of VA proved in the present study as well as in a previous *invitro* study of **prince *et al*. (2011)** showing the scavenging effect of VA on the superoxide radical. Therefore, the protective effect of VA may also due to scavenging superoxide anion, which reacts with NO to form the harmful radical peroxynitrite. Thus, VA may help to maintain a protective balance between NO versus ROS production, which is vital to the prevention and resolution of ALF.

## 5. CONCLUSION

Vanillic acid elicited a dose-dependent protection against acute liver failure induced by thioacetamide in rats. This protection may be due to its antioxidant and anti-inflammatory properties.

### *6. Declaration of interest*

The authors report no declarations of interest.

## ACKNOWLEDGEMENTS

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